Role of 11β-hydroxysteroid dehydrogenase 1 on sebocyte differentiation

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11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) is an enzyme involved in glucocorticoid regulation through the catalysis of the conversion of inactive cortisone to its active form cortisol, thereby amplifying the intracellular glucocorticoid action. Many studies have been conducted over the last decade into the key role of 11β-HSD1 in lipid and glucose metabolism and into the potential use of 11β-HSD1 inhibitors for the treatment of the metabolic syndrome. However, the function of the enzymatic activity in skin is not known. Here we review recent evidence suggesting the function of 11β-HSD1 in sebaceous glands. Using PCR and immunohistochemistry in human skin, we confirmed that 11β-HSD1 (which activates cortisol), contrary to 11β-HSD2 (which inactivates cortisol), is preferentially expressed in human sebaceous glands. We investigated the direct role of 11β-HSD1 in lipid synthesis using cortisone-stimulated cultured primary human and rat sebaceous cells and the use of reference or new pharmacological inhibitors of the enzyme. We demonstrated that cortisone stimulates neutral lipid neosynthesis and that 11β-HSD1 inhibitors inhibit stimulated lipid synthesis. These same compounds were evaluated in vivo and completely reversed the cortisone-induced rise of skin surface lipids after topical administration. Conclusion: Pharmacological inhibition of 11β-HSD1 in sebaceous cells prevents the effect of cortisone on lipid synthesis. Hormonal regulation of glucocorticoid action in sebaceous glands might be relevant as a treatment of dermatological diseases such as acne.

Full-thickness skin with mature and cycling hair follicles using tissue culture expanded human cells

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Regeneration of hair follicles utilizing tissue cultured expanded human dermal and epidermal cells has been a long-term challenge. Although a recent report described human hair follicle formation within a skin equivalent containing TSCII mutant skin fibroblast cells, the hair shafts formed were abnormal. Here, we report the formation of skin with robust hair follicle growth using dissociated and cultured human scalp dermal cells combined with cultured neonatal foreskin keratinocytes. Our results show a full-thickness skin containing mature epidermis, dermis as well as subcutis. The regenerated hair follicles show normally layered sheaths with sebaceous glands, arrector pili muscles, dermal papilla, and mature hair shafts. The follicles show anagen, catagen and telogen forms. We demonstrated that the regenerated skin and the hair follicles therein (including interfolliclular and follicular epidermis, dermal fibroblasts, papilla and dermal sheath) are of human origin. Staining with differentiation markers documented good follicular and interfollicular epidermal maturation and regenerated interfollicular and follicular epidermis with distinct differentiated layers. Interestingly, human antigen positive sweat glands, vessels and neurons were present in the skin grafts, a novel finding. Time-course analysis shows that neogenesis of human hair follicles recapitulates embryonic morphogenesis. Finally, we show that the reconstituted skin can maintain human features for at least 1 year after grafting, and that it is able to heal with human cells after injury. In summary, our findings have four implications: 1. Tissue cultured expanded human cells can indeed produce mature skin with subcutis and fully developed cycling, shaft-forming hair follicles and eccrine glands; 2. Closer study of this system will allow mechanistic insights into human skin and appendage physiology and morphogenesis; 3. This system will allow for new laboratory models of skin disorders using patient or mutated cells and, 4. The method will accelerate the generation of a complete skin for clinical applications.

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Sphingolipids of murine hair: Biochemical and immunohistochemical analyses

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Recent interest in hair follicle biology has revealed a complexity of cell types and signal pathways in forming the hair shaft. For controlling hair cycling, lipids also play important roles. Among them sphingolipids are expected to participate in hair functions/cycles as components and signaling molecules, however, there have been few reports until now. In this research, we analyzed hair sphingolipids and revealed that murine hair sphingolipids were different from those of epidermal sphingolipids. In wild murine hair, there are ceramide (Cer), glucosylceramide (GlcCer) and sphingomyelin (SM). Cer of murine hair has no acylCer which is a key molecule of the epidermal barrier, and a small amount of Cer with alpha-hydroxy (OH) fatty acids (FA) which are rich in the epidermis. The major molecule of murine hair Cer was C20:0/d18:0. Both whole hairs (including follicles) and hair shafts have GlcCer with little or no acylGlcCer. The major FA of hair GlcCer were C18 molecules. GlcCer carrying long chain alpha-OHFA (chain length more than 20) were detected in whole hairs, whereas these alpha-OHGlcCer were rarely detected in the shaft. Whole hairs have SM but hair shafts have little SM, indicating that SM is a component of hair follicular cells. The major FA of hair SM were C16:0, C18:0, C24:0 and C16 alpha-OH:0. Immunohistochemical studies showed that the hair shaft was stained by anti-GlcCer antibody while SM, which was recognized by lysenine, was stained in the root sheath cells but not in the hair shaft. GlcCer seemed to be expressed strongly in inner root sheaths and weak in outer root sheaths, while, SM distributed diffusely in the outer root sheaths of the follicles. In hairs, the metabolic pathways and functions of GlcCer and SM may be different from each other.

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Screening for autoantigen epitopes involved in the development of alopecia areata

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The development of alopecia areata (AA) is believed to involve an autoimmune mechanism. In both humans and rodent models, AA is a non-scarring, inflammatory hair loss disease where CD4 and CD8 (CTL) T cells are required for the onset and progression of AA. Hair follicle (HF) antigens derived from keratinocytes and/or melanocytes have been suggested to be able to trigger auto-reactive CTL response in AA subjects, but the exact epitope targets are not yet identified. We investigated the potential of a panel of epitopes expressed by human HF keratinocytes and melanocytes to induce activation of CTLs. Peripheral blood mononuclear cell (PBMC) populations were isolated from AA and healthy subjects with HLA-A2 serotypes. Synthesized HLA-A2 restricted peptides with sequences specific for trichohyalin, melanin, MART1, tyrosinase, tyrosinase related protein-2 (TRP2) and GP-100 were cultured with PBMCs. The frequency of CTL activation in PBMC was measured by using enzyme-linked immunosorbent spot (ELISpot) assays where activated IFNgamma secreting cells are visible as spots. Epitope peptide cocktails derived from trichohyalin, TRP2 and MART1 induced significantly higher CTL responses in AA subjects. Investigation into CTL activation via single trichohyalin epitopes showed highly variable results, suggesting patients with different stages of AA may have different primary epitope targets. AA affected C3H/HeJ mouse lymph node cells (LNCs) showed significantly higher responses to mouse antigen epitopes like keratin-16 (K16) and MART1 but less so to trichohyalin; unlike in human PBMCs. The data indicate that AA affected subjects and C3H/HeJ mice have PBMC populations with an increased frequency of CTLs responsive to antigen epitopes originating from keratinocytes and melanocytes compared to their respective healthy controls. Potentially, trichohyalin, MART1 and K16 could be specific targets for CTLs that cause AA.

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Validation of a high throughput human primary sebocyte discovery platform

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The discovery of new treatments for acne is impeded by the limitations of current sebaceous gland and sebocyte model systems. For the rapid assessment of novel compounds, natural products or new formulations, researchers rely on the use rodent cells or immortalized cell lines. While being extremely useful, these cells have limitations with regard to human primary cells and clinical outcomes. Historically, the isolation, proliferation and maintenance of human primary sebocytes in culture have proven to be extremely difficult. Their limited availability and requirement for larger format assays has slowed the pace of Human Primary Sebocyte (HPS) research and drug discovery. We sought to determine if we could miniaturize an HPS platform for high throughput parallel analysis of lipid accumulation, lipid synthesis and viability in a 96-well format. HPS isolated from different donors, using facial sebaceous glands derived from surgical waste are expanded in the absence of a feeder-layer and cryopreserved. HPS are seeded in 96-well plates and induced to differentiate. As expected, after differentiation, HPS treated with insulin, IGF-1 or T0901317 (LXR agonist) for 24-72 hours exhibit a significant increase in lipid accumulation and synthesis as determined by Nile Red staining and 14C acetate labeling. In addition, inhibitors, such as 13-cis retinoic acid potently inhibit this response. We have incorporated an automatable lipid extraction procedure which allows higher throughput processing to assess lipid synthesis activities. To obtain a detailed lipid composition, we have performed TLC separation and a quantification of label incorporated into each species (triglyceride, squalene, cholesterol esters). Our results show that this platform can be a useful tool for screening compound libraries or detailed dose response studies of lead compounds or natural products. To this end we screened a library containing 580 botanical extracts to identify modulators of lipid synthesis. These results are compared to a previous screen for effects in human adipocytes using the same library.

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Tissue engineering a 3-D functional model of the human eccrine sweat gland

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weat gland (ESG) is highly important in thermoregulation, homeostasis and contributes to maintaining electrolyte balances. Full thickness burns damage the ESG and even after recovery they are not regenerated, making burns patients susceptible to hyperthermia and heat stroke. Even though ESGs are essential for thermal regulation and implicated in conditions such as cystic fibrosis, the mechanisms of its function have yet to be fully understood. A 3D histotypic model of the ESG would be a valuable tool for studying its function, morphogenesis, pharmacological interactions and as a platform for the development of a fully functioning tissue engineered skin substitute. In this study, we have immortalized human eccrine cells from the secretory coil by using HPV16E6E7. From these cells we derived 9 clones which together with the original cell line (EC23) we characterized with a panel antibodies specific for secretory coil cells, to help us differentiate populations of clear and dark cells. We have also identified nestin and beta1-integrin expression on cells within our cell line, suggesting there may be a stem cell niche within these cells, and as a result could have a great regenerative potential in 3D cultures. EC23 cells were also examined for their response to the cholinergic agonist carbachol. Extracellular calcium is essential for its normal function. Calcium flux responses on cells deprived from calcium and supplemented with 1uM calcium chloride were assed, providing evidence of the retained functionality of these cells after immortalization. Furthermore, collagen gel organotypic raft cultures containing fibroblasts, immortalized eccrine cells and primary keratinocytes were cultured for 14 & 21 days. Our initial findings suggest that EC23 cells have the potential of regenerating ESG in collagen organotypic models.

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Thyroid hormones regulate key parameters of mitochondrial biology in human skin epithelium *in situ* and may exert anti-ageing effects

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The thyroid hormones (THs) triiodothyronine (T3) and thyroxine (T4) are potent regulators of mitochondrial biology; Thyroid gland dysfunction often co-exists with skin and hair abnormalities. However, the effects of THs on mitochondrial biology in human skin are largely unknown. Therefore, we characterized these effects in organ-cultured human skin. We show that T3 (100 pM) and T4 (100 nM) up-regulate the immunoreactivity (IR) of the mitochondrially-encoded cytochrome c oxidase I (MTCO1) ($p<0.001$) and increase complex I activity ($p<0.001$), whilst only T3 up-regulates mitochondrial transcription factor A (TFAM) IR ($p<0.001$). By electron microscopy we show that T3 and T4 increase the number of perinuclear mitochondria in epidermal keratinocytes ($p<0.05$). Given that mitochondrial stimulation can result in both beneficial (e.g. increased energy supply, anti-ageing) and undesirable effects (e.g. increased DNA damage by enhanced ROS production, leading to premature tissue ageing), we also investigated the effects of THs on specific ageing parameters after 24 hrs or 6 days of treatment: matrix metalloproteases (MMPs)-1, -2 and -9 (up-regulated in ageing skin); fibrillin-rich microfibrils, and collagens I and III (down regulated in ageing skin). Interestingly, MMP-1 protein expression was down-regulated by T3 ($p<0.001$) and T4 ($p<0.01$) after 24hrs, while the activity of MMPs -1, -2 and -9 and collagens I and III IR appeared largely unaffected. Fibrillin-rich microfibrils were deposited in the papillary dermis in response to TH culture. These findings provide the first evidence that T3 and T4 are strong endocrine stimulators of mitochondrial activity and biogenesis in human epidermis in situ and suggest that THs may also exert some anti-ageing properties, which could be related at least in part to the mitochondrial effects of THs.

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11β-HSD1 inhibitor reverses the overproduction of sebum induced by topical application of cortisone in rat skin

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The presence of acne-like lesions is a common side effect of oral and topical glucocorticoid therapy. 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) is a primary regulator of glucocorticoids catalysing the reduction of inactive cortisone to its active form cortisol. 11β-HSD11 is an NADPH-dependent enzyme highly expressed in key metabolic tissues including liver, adipose tissue, and the central nervous system. In human skin, 11β-HSD1 is present in keratinocytes and fibroblasts and has been recently described to be expressed in sebaceous glands although the function of 11β-HSD1 in the skin and in the acne pathology remains unknown. The role and the activity of 11β-HSD1 on sebaceous gland homeostasis were investigated in female SD hairless rats. Rats were topically treated with cortisone in combination with a potent and selective inhibitor of the enzyme. Using immunohistochemistry, we showed that 11β-HSD1 is preferentially expressed in the sebaceous glands. Topical administration of cortisone strongly increased both the cortisol level in female rats and the quantity of lipids present at the skin surface. In parallel, this increase was accompanied by a modification of the lipid composition of the sebum. At all tested doses, 11β-HSD1 inhibitor was able to reverse the cortisone-induced modification of the sebum (at both quantity and composition level) and to inhibit the rise in the skin cortisol rate. Our results clearly suggest that 11β-HSD1 locally regulates the glucocorticoid-dependent homeostasis of sebaceous gland and that 11β-HSD1 inhibitors might have an interest in acne therapy.

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The roles and capabilities of multipotent nestin-expressing stem cells of the hair follicle

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Nestin-expressing hair follicle stem cells of the mouse have been previously shown to differentiate into neurons, glia, keratinocytes, smooth muscle cells and melanocytes in vitro. Nestin-expressing hair follicle stem cells enhanced the rate of nerve regeneration and the restoration of nerve function after transplantation in mouse models. The nestin-expressing hair follicle stem cells transdifferentiate largely into Schwann cells when implanted in severed nerves or injured spinal cord, which may enhance neuron regrowth. Nestin-expressing stem cells can also be readily isolated from the human scalp. The question remains as what is the in situ role of the nestin-expressing cells in the hair follicle. To attempt to answer this questions, the vibrissa hair follicles including their sensory nerve stump were excised from transgenic mice in which the nestin promoter drives green fluorescent protein (ND-GFP) and were placed in 3D culture supported by Gelfoam®. The nestin-expressing cells trafficked from the bulge area to the whisker sensory nerve stump over a 21-day period in histoculture. ND-GFP expressing cord-like structures extended from the nerve stump which consisted of ND-GFP-expressing spindle-shaped cells, which co-expressed the neuron marker β-III tubulin, the immature Schwann-cell marker p75NTR and TrkB which is associated with neurons. The fibers extended to at least 500 μm from the whisker nerve stump in Gelfoam® histoculture and had growth cones on their tips expressing F-actin suggesting they were growing axons. The extending whisker sensory nerve was highly enriched in ND-GFP cells which appeared to play a major role in its elongation and joining with other nerves in 3D culture.

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Timing the hair cycle: BMAL1 knock-out induces anagen prolongation and increases melanogenesis in the hair follicle

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The human hair follicle (hHF) is an easily accessible model organ, which undergoes lifelong cyclic behavior, oscillating between an active growth phase (anagen) a regressive phase (catagen) and a phase of relative quiescence (telogen). While many candidate molecules have been identified that modulate the hair cycle, the underlying autonomous oscillator system remains to be elucidated. In murine skin, core clock genes (CLOCK, BMAL1, PER and CRY) oscillate in phase with the hair cycle. Also, preliminary evidence suggests that this occurs also in hHFs and that knock-down of PER1 prolongs anagen and stimulates melanogenesis in cultured hHFs. However, it currently remains unknown whether these clock gene silencing effects are PER1 specific or are caused by a disruption of the molecular clock. As BMAL1 induces transcription of PER1 and its knock-down disrupts the molecular clock, we addressed this question by BMAL1 silencing with specific siRNA in hHF organ culture. Quantitative histomorphometry showed that BMAL1 knock-down HFs remain significantly longer in anagen VI than control HFs transfected with a random oligonucleotide (42% versus 10% after 4 days, $p=0.028$, $n=40$ from 3 patients), and HFs showed an increased percentage of ki-67 positive in the matrix keratinocytes in the BMAL1 knock-down HFs (41.6%) when compared to the control group (33.9%). Furthermore, mirroring the effect seen by PER1 knock-down, BMAL1 silencing significantly increased the melanin content of anagen HFs ($p=0.014$, $n=16$ from 3 patients). These data suggest that the HF is indeed a peripheral clock oscillator and BMAL1 is a new modulator of human hair pigmentation.

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Learning your ABCs: xenobiotic transporter expression in human hair follicles

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ATP-binding cassette (ABC) transporters fulfil a crucial role in xenobiotic defence across a range of species and tissues. Past research has identified their prominent role in reducing the accumulation of toxins within cells. Certain members of this superfamily, including ABCB1 (P-glycoprotein) and ABCG2 (Breast cancer resistance protein; BCRP) are also expressed in various stem cell populations, conferring the side-population phenotype by exclusion of the dye, Hoechst 33342 (H33342). Currently, expression and function of these membrane transporters in the human hair follicle are unknown. We utilised ABC transporter TaqMan arrays to characterise the expression of their RNA in isolated human hair follicles from 2 male patients. Protein expression and localisation of stem-cell associated and xenobiotic defence ABC transporters (ABCB1, ABCG2, ABCC4 and ABCB5) was examined by immunofluorescent staining. Strong immunoreactivity for ABCB1 was noted in the proximal IRS and terminally differentiating keratinocytes of the bulb. ABCC4 immunofluorescence was particularly prominent throughout the ORS and IRS of the proximal HF, yet absent in the stem-cell-containing bulge. Instead, ABCG2 protein was predominantly expressed in matrix keratinocytes and the bulge region and co-localised with the HF stem cell marker keratin 15. ABCB5 expression was restricted to the ORS of the lower hair follicle. Functional ABCG2 efflux activity was assessed intra-vitally via 30 minutes incubation with the ABCG2 substrate H33342, in the presence and absence of the specific ABCG2 inhibitor Ko143. Application of the inhibitor increased dye accumulation in the bulge region. These data provide the first evidence for expression and functionality of multiple ABC transporters within defined compartments of human hair follicle epithelium, including the stem cell region. ABCG2 may contribute to the protection of stem cells against toxic insult such as chemotherapy.

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***In vitro study* of the effect of glycation on an innervated and endothelialized tissue-engineered skin**

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Diabetes is a chronic disease inducing blood hyperglycemia leading to vascular diseases, skin problems, diabetic neuropathy, and death. Hyperglycemia induces direct adverse effects on cells, but also promotes the formation of advanced glycation end products (AGEs) that could participate to the disease phenotype. AGEs are formed from a high glucose level leading to an increase of its oxydative breakdown products such as 3-deoxyglucosone (3-DG), glyoxal and methylglyoxal. We previously developed a tridimensional tissue-engineered skin made of a collagen sponge biomaterial cultured with fibroblasts, endothelial cells, keratinocytes and innervated with sensory neurons. This reconstructed skin combines a microvascular and a nerve networks. To discriminate between hyperglycemia and glycation in the analysis of diabetes-induced skin neuropathy and impaired vascularization, we report here the glycation of this model using high concentration of ribose or glyoxal. We first showed that glycation induces expression of carboxymethyl lysin (CML, an AGE product) and vimentin relocalization in fibroblasts cultured in monolayer. Treatment of sensory neurons with glyoxal induced toxicity in a dose dependant manner, and intracellular CML expression with an increase of endoplasmic reticulum stress showed by an increase of GRP78. The addition with glyoxal of aminoguanidine, a glycation inhibitor, prevented CML formation in both cell types. We also showed CML expression in our tissue-engineered skin, where cells can sustain much higher glyoxal concentrations than in monolayer cultures. Thus, this glycated model of an endothelialized and innervated tissue-engineered skin will be a useful tool to better understand the effects of glycation on capillaries and nerves, and to screen new deglycation molecules for future therapeutic treatments.

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Soy extract modified by biotechnology increases synthesis of a functional characterized heterotypic type I/V/III collagen fibers network in the extracellular matrix of the human skin

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Collagen is a major structural protein responsible for skin firmness. With ageing collagen synthesis decreases due to a loss in cell metabolism and increase of catabolism. The classical anti-ageing approach consists of stimulating the major collagen (collagen I) by increasing its quantity. However 3D organization and functionality are progressively modified. We decided to stimulate collagen I in conjunction with collagen V to help newly synthesized collagen I molecules to be correctly organized into fibrils in the dermis. An in-house screening model has been developed to measure collagen synthesis in different cell compartments of fibroblast cell culture monolayers, after different steps: measure of procollagen precursor form in the extracellular medium, cellular lysis to obtain a matrix access, cell lysate recovery to perform DNA assay, and measure of collagen I and V deposition in the matrix. This model shows that a soy extract stimulates procollagen precursor synthesis and collagens Type I and V deposition by respectively 22.9% and 51.3% versus the non-treated condition. Collagen I immunostaining was also performed and analyzed by confocal microscopy and show increase of type I and type V collagen fibers, equivalent to the positive control vitamin C, respectively +43% and +38% preserving physiological ratio. Moreover, the orientation of collagen fibers obtained with soy extract shows a real entangled network compared to the longitudinal orientation obtained with the wound healing marker TGF-beta. Finally, images obtained by TEM show that soy extract increases synthesis of a functional heterotypic I/V/III collagen fibers by the observation of characteristic striations of such fibrillar collagen network. These in vitro results provide new evidence of the ability of soy extract modified by biotechnology to increase synthesis of a functional collagen fibers network to improve skin firmness.

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Ajuga reptans enriched in 50% of phenylpropanoids: New evidences for hair disorders

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Free radicals and subsequently oxidative stress, are implicated into different physiological mechanisms such as senescence, inflammation, hair graying and hair loss, so the antioxidant treatment can help ward off damage. In this study Ajuga reptans titrated at 50% in phenylpropanoids was investigated for its antioxidant activity by DPPH assay, in vitro tests on fibroblast cells, studying the protection against peroxide-hydrogen induced oxidative stress and ROS production in DCFH-DA assay. The modulation of 5 alpha-reducatse (SRD5A) acitvity was also investigated by qRT-PCR and Elisa assay. All the test were performed vs wild Ajuga reptans extract. Compared to the wild extract, Ajuga reptans titrated at 50% in phenylpropanoids exerted a significative and dose-dependent scavengers activity against the DPPH free radical. These data were confirmed by both in vitro assays. Effect of Ajuga reptans titrated at 50% in phenylpropanoids against induced oxidative stresses was higher than the wild extract and it was due to the strong inhibition of intracellular ROS production. Data from gene expression of SRD5A also showed that Ajuga reptans 50% titrated in phenylpropanoids exerted an inhibitory effect towards 5 alpha-reductase enzyme by its strong selective down regulation on 5aR2 isoform gene expression. No 5aR2 inhibition was reported for wild extract. In conclusion, Ajuga reptans with its high antioxidant activity and the inhibition of 5α-reductase enzyme could represent a good candidate for hair disorders treatment.

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Muscarinic agonist induction control of sweat glands

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Eccrine glands are a significant means of thermoregulation and heat dissipation in humans. When the body's internal temperature exceeds a set point by exercise or heat-induction, a sympathetic reflex is activated resulting in general sweating, vasodilatation and increased breathing. Stimulatory effects of both cholinergic and adrenergic pathways can evoke sweating response, but adrenergic stimulation is significantly different. Secretory rates of cholinergic agonists are several magnitudes larger than adrenergic agonists. Both types of agonists have been shown to be less uniform in rapidly evoking sweating. Skin, ex vivo, retains the ability to respond to a variety of secretagogues by receptor mediated processes including muscarinic agonists. Muscarinic receptors are located in the smooth muscles of the blood vessels, heart, lungs, as well as exocrine glands. These receptors, when reacted with muscarinic agonists, help stimulate secretion in eccrine glands. Our aim was to develop a model to evaluate the efficacy of muscarinic agonists with expected sweat-inducing properties in human skin explants, where drug concentrations, temperature, and time are readily controlled. The study was performed using tissue from several donors where the subcutaneous adipose tissue was removed and the resulting tissue was mounted in a free flowing chamber. Sweating was induced by several different muscarinic agonists, vehicle controls, and sham compounds. The degree of sweating with the different drug applications was determined by measurement of transepidermal water loss and by starch/iodide method. No statistically significant differences were found in the TEWL between vehicle and sham. In all cases, where treatment with the muscarinic agonist was included, significant sweating occurred as measured by TEWL and starch/iodide. This present model mimics sweating in a similar manner in vivo. We suggest the feasibility of using this model as a tool for testing the efficacy of drugs, antiperspirants, and sweating disorders.

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A novel strategy for therapeutic hair growth inhibition: PPARγ modulation promotes catagen and induces hair matrix apoptosis while inhibiting inflammation and preserving the epithelial stem cell compartment

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Inhibition of undesired hair growth (e.g. hirsutism) remains a major clinical challenge. Signaling through peroxisome proliferator-activated receptor-gamma (PPARγ) exerts anti-inflammatory effects, modulates keratinocyte and sebocyte differentiation and is needed for preserving hair follicle (HF) epithelial stem cells (PPARγ signaling is defective in lichen planopilaris). Therefore, we have investigated how a novel agonistic PPARγ modulator, GMG-43AC, impacts on human hair growth (0.01-1mM GMG-43AC, 6d HF organ-culture), and modulates TNF-α- or IFN-γ induced IL-6 mRNA/protein expression (0.5mM GMG-43AC, 6 or 24h) of primary human epidermal keratinocytes (NHKs) in vitro. Despite large interindividual variations in the HF response of 6 tested female patients to GMG-43AC, overall, 1mM of GMG-43AC inhibited hair shaft elongation, significantly enhanced catagen development and increased hair matrix keratinocyte apoptosis ($p<0.05$). Intriguingly, GMG-43AC also up-regulated expression of the epithelial progenitor cell keratins, K15 and K19. Microarray analysis revealed several intrafollicular candidate GMG-43AC target genes that invite subsequent mechanistic studies. GMG-43AC also inhibited IL-6 gene and protein expression of NHKs induced by pro-inflammatory cytokines. Given its favourable toxicological profile, anti-inflammatory and hair growth-inhibitory properties, this makes GMG-43AC an interesting novel candidate anti-hirsutism agent (e.g. topical application after standard depilation). That GMG-43AC also stimulates HF epithelial stem cells may help to preserve the HF's regeneration potential and also invites exploration of this novel PPARγ modulator in lichen planopilaris.

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Calcium-dependent deimination, followed by zinc-involved tetramerization, of S100A3 during the trichocytic dfferentiation

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Mature hair cuticles form the outermost protective tissue of the hair fiber. Hair cuticle constitutes the cornified envelope thicker than that of skin corneocytes; however, its terminal differentiation process remains unclear. In human hair cuticular cells, a hair dominant type of Ca2+-dependent peptidylargine deiminase (PADI3) catalyze the conversion of specific arginines on the homodimer interface of S100A3 into citrullines. This irreversible modification causes assembly of an S100A3 homotetramer in the presence of Ca2+ and Zn2+. Phylogenetic analysis suggests that divergence of the S100A3 gene coincided with the emergence of hair, a defining feature of mammals. Amino acid sequences deduced from therian S100A3 genes conserve the (Cys)3His-type Zn2+-binding site in the C-terminus in addition to two EF-hand-type Ca2+-binding motifs. To elucidate functional significances of Ca2+- and Zn2+-homeostatic regulation underlying in the superficial epithelium, the structural and functional role of the C-terminal Zn2+-binding domain in the S100A3 tetramrerization were investigated. The binding of either Ca2+ to two EF-hand-type Ca2+-binding motifs or Zn2+ to the (Cys)3His-type Zn2+-binding site reduced the α-helix content. The binding of a single Zn2+ cation promoted Ca2+-dependent tetramerization of S100A3 and induced extensive unfolding of helix IV. The Ca2+ and Zn2+ binding affinities of S100A3 were enhanced by binding of the other cation in conjunction with the tetramerization. Binding of Ca2+ or Zn2+ to each S100A3 subunit within the homotetramer is induced by repositioning of helix III and rearrangement of the C-terminal tail domain. The heterotrophic allosteric modulation of S100A3 by binding of Ca2+/Zn2+ suggests that S100A3 is involved in Ca2+- and Zn2+-homeostasis in the superficial epithelium.

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Communicate to grow: The example of the human hair follicle

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The hair follicle (HF) is a highly organized system that undergoes successive phases of intense activity and resting period, separated by transition phases. Due to its unique organization and functioning, the HF is the seat of numerous interactions that take place inside the HF itself and between the HF and its cutaneous environment. In the present study, we were interested in studying the interactions implicating laminin-511 (LN-511), its integrin receptor and β-catenin. For this purpose, we developed a specific compound that targets LN-511. The first part of the study consisted in characterizing the expression of LN-511 and α3- and β1-integrins in cultured normal human keratinocytes (NHK) and dermal papilla cells (DPC). Then, in order to study communication between these two cell types, we applied conditioned medium of NHK treated with the selected compound on DPC. We observed an increase in β-catenin staining in DPC treated with NHK-conditioned medium, suggesting an effect on intercellular communication. The outcomes of the treatment were then studied at the level of the hair follicle by using two models: ex vivo scalp biopsies and isolated hair follicles. A 24h application of the compound led to an increase in the staining of LN-511 and α3- and β1-integrins in the hair follicles of both models. Moreover, in hair follicle maintained in culture for a longer time, the compound helped preserve LN-511 expression and HF structure. LN-511, its integrin receptor and β-catenin were previously associated with hair follicle maintenance in the active phase of the hair cycle (anagen). By helping maintain their level inside the HF and HF cells, the compound targeting LN-511 could be considered as a potential candidate for preservation of optimal hair growth environment.

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Different approaches to assess the effects of stress on human hair follicle

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The hair follicle (HF) is constantly exposed to multiple stressors, from external and internal origins, resulting in modifications of hair final appearance. In the present study, our aim was to develop in vitro and ex vivo models, in order to assess the effects of different stresses at the level of HF and HF cells. The first model consisted in full-thickness biopsies of scalp skin, maintained in an air-liquid interface culture system. This model preserved the interactions between the HF and its cutaneous environment and allowed topical application of potential stressors or formulated products. By performing serial sections at the level of the HF followed by hematoxylin-eosin staining, we observed the damaging effects in the hair bulb, resulting from the topical application of a detergent solution. Moreover, this ex vivo model was also appropriate to the evaluation of damage induced by UVA/UVB irradiation and oxidative stress. The second model used was the HF isolated from scalp skin by microdissection. When exposed to H2O2 stress, a reduction of melanin content was observed in the hair bulb, as revealed by Fontana-Masson staining. Finally, using fibroblasts from dermal papilla cells, we developed a model of in vitro aged human dermal papilla cells (HDPC) by applying methylglyoxal (MGO). The aged phenotype of MGO-stressed HDPC was confirmed by altered cell morphology, increased beta-galactosidase staining, reduced alkaline phosphatase activity and disorganized fibronectin network. Furthermore, electron microscopy revealed vacuolization and decrease in intracellular organelles in stress-induced senescent cells. Different approaches were developed to evaluate the effect of stress on HF, from the most complete full-thickness scalp biopsies to isolated HF and cultured HF cells. Combinations of these different methods could help understand HF response to different stressors, in order to develop solutions for HF protection.

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Transcriptome analysis of estrogen regulating factors in hair cycle

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Female pattern hair loss (FPHL) is a distinctively baldness in women and the prevalence increases with aging. But there are few reports concerning FPHL as compared to male pattern hair loss. Generally, the onset of FPHL is affected by various factors and estrogen is considered one of the responsible factors, but the detail remains to be elucidated. Therefore we examined the effects of estrogen on dermal papilla cells (DPCs) and hair cycle in ovariectomized (OVX) mice to reveal the mechanism of FPHL particulars. Firstly, we cultured human DPCs derived from female hair follicle in DMEM medium and treated with 10nM estradiol. Total RNA was isolated 4h after estradiol addition, and we analyzed genome-wide expression data obtained from DNA microarray experiment. In the presence of estradiol, 2,555 gene expressions were increased more than two-fold or decreased less than half, in 21,558 genes subjected to this analysis. We could select 203 genes, such as BMPs, Bcl2 and LEF1, from them based on the literatures on the hair functions. Next, we compared hair cycle of OVX mice with that of sham-operated (Sham) mice. Female C57BL/6N mice (3 wk old) were operated, and depilated to induce anagen at the age of 10 weeks. We observed the disorder of hair cycle in OVX mice. Furthermore, immunohistological analyses of dorsal skins revealed that some proteins, such as LEF1, which caused variations of gene expression levels in DPCs by estradiol, existed around hair follicle and associated with hair cycle, were down-regulated in OVX mice compared to that in Sham mice. We also evaluated several ingredients, which were provided enough evidence of effectiveness such as 6-benzylaminopurine (6-BA). We found that 6-BA could up-regulate the gene expression levels of BMP2, Bcl2 and LEF1, and could recover the changes which were caused without estradiol in DPCs using qRT-PCR. Our findings indicate that estrogen affects hair cycle via the mechanism including some gene expression changes such as BMP signaling.

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Analysis of hair shaft cuticle-specific keratin-associated protein 10 (KRTAP10) family

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Hair is a strongly keratinized tissue formed within the hair follicle. The major structural components of the hair shaft are hair keratins and their associated proteins (KRTAPs). To date, more than 80 *KRTAP* genes have been identified in humans, which are classified into a total of 27 families based on their sequence homology and the nature of the repeat structures. Although KRTAPs are believed to interact with hair keratins and contribute to form a rigid hair shaft, their biophysical features remain largely unknown. We recently reported the characteristics of hair shaft cortex-specific KRTAP2 family members at the DNA, RNA, and protein levels. In this study, we focused on hair shaft cuticle-specific KRTAP10 family members. We confirmed the expression of KRTAP10 proteins in upper keratinizing zone of the hair shaft cuticle by indirect immunofluorescence (IIF) with an anti-KRTAP10 antibody. A series of *in vitro* studies demonstrated that the KRTAP10 proteins interacted with each other, and also showed affinity to cuticle-specific hair keratins, such as K82 and K32. Furthermore we performed IIF studies in cultured cells to show co-localization between KRTAP10 proteins and hair keratins in the cytoplasm. Our data provide crucial information to understand the mechanisms of keratinization of the hair shaft cuticle.

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A novel heterozygous deletion-insertion mutation in the mouse *Krt71* gene underlies wavy coat phenotype

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We have recently reported that a heterozygous mutation in the inner root sheath (IRS) – specific keratin 71 (*KRT71*) gene underlies autosomal dominant woolly hair in humans. We herein analyzed a novel mouse mutant strain displaying a wavy pelage and curly vibrissae that has arisen in a colony of C57BL/6 mice. The mutation was identified as a heterozygous deletion-insertion mutation c.1117_1123delinsAGCCTTCTAT in exon7 of the *Krt71* gene, which was predicted to affect the codons for Leucine 373 to Threonine 375, substituting them for a codon for Serine, Leucine, Leucine and Serine (p.L373_T375delinsSLLS). This mutation is not located within, but in the vicinity of the helix termination motif (HTM) of K71 protein. The morphologic analyses demonstrated that the mutation caused the formation of filamentous aggregates in the IRS of the hair follicle, leading to the bending of the hair shaft. Immunohistological analysis revealed an abnormal immunoreactivity with an anti-K71 antibody in these mice. We also demonstrated that the mutant K71 protein led to minimal disruption of keratin intermediate filament (KIF) formation in HaCaT cells. Our results further underscore the crucial role of K71 in keratinization of the IRS across species.

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Inhibitory effects of anti-histamines against T cell-chemoattractions by suppression of F-actin polymerization in alopecia areata

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Alopecia areata (AA) is considered as a tissue specific autoimmune disease by T cell-mediated autoimmune reactions. Although detailed pathomechanisms still remains to be elucidated, we have reported that the accumulation of Th1/Tc1 cells around hair bulbs (so called swarm of bees) is induced by increased expression of CXCL10 in AA lesions. In addition, we observed the upregulation of Th1 and Tc1 cell velocity toward CXCL10 in PBMCs from AA patients. In Japanese guideline for the management of AA, antihistamine drugs can be considered for use, but there has been insufficient evidence. In this study, real-time and horizontal chemotaxis assay (EZTaxiscan) was demonstrated with PBMCs obtained from AA patients. CD4+ and CD8+ T cells from AA showed significantly higher velocity toward CXCL10 compared to that of healthy subjects as reported before. However, pre-culturing of PBMCs with olopatadine for 8 hours decreased the velocity. Although CXCR3 and CCR4 expression on T cells were no remarkable changes, F-actin polymerization assay showed decreasing the phalloidin binding to polymeric and oligomeric forms of actin by the treatment with olopatadine. Furthermore, Ca-influx was also suppressed in PBMCs from AA patients by olopatadine treatment. In conclusion, antihistamine, olopatadine, may inhibit chemotactic activity by suppression of F-actin polymerization and Ca-influx in CD4+/CD8+ T cells from AA patients that indicate intervention of T cell accumulation around hair bulbs. These results strongly support the recommendation of anti-histamine drugs to apply on the treatment of AA.

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Epithelial deletion of Wntless causes premature regression of hair follicles and epidermal hyperplasia

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Wntless (Wls) is required for the secretion of Wnt ligands in multiple tissues and organs. The function of Wls in skin biology is being elucidated, yet far from clearly defined. Here, we deleted Wls in mice epithelium with a hypomorphic K14-cre. The Wls cKO mutants displayed wavy and patchy hairs due to partial failure of hair follicle initiation. The remaining hair follicles were well developed but prematurely regressed. Once entering telogen phase, these follicles hardly re-entered the subsequent anagen phase and progressively became malformed, finally degraded to dermal cysts. In addition, the epidermis of postnatal Wls cKO mice were significantly expanded, characterized with ectopic expression of basal genes in suprabasal layers. The epidermal phenotype of Wls cKO mice showed similarity with that of Wnt5a null mice, implicating Wls-mediated non-canonical Wnt signaling may regulate the homeostasis of postnatal epidermis. Thus, epithelial Wls is required for proper postnatal hair cycling and epidermal homeostasis.

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Human sebocytes treated with ROCK inhibitors produce lipids in the absence of mature sterol response element binding protein (SREBP)

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Model systems to study lipid production in human sebocytes are of interest in evaluation of novel therapeutic agents in acne. Insulin increases lipid production in SEB-1 sebocytes (immortalized by SV40) via increased expression of mature sterol response element binding protein (SREBP1). Recent studies indicate that primary sebocytes and keratinocytes can be expanded indefinitely in culture when grown in the presence of Y-27632, an inhibitor of Rho-associated protein kinase (ROCK). The goal of this study was to examine the role of SREBP in mediating lipid production in human sebocytes maintained in the presence of ROCK inhibition but not immortalized (P-SEB) and sebocytes spontaneously immortalized in the presence of ROCK inhibition (Y-SEB) and to compare these with data in SEB-1. Using 14C-acetate incorporation, all 3 cell types produce characteristic sebaceous lipids. Following removal of Y-27632, P-SEB increases lipid production indicative of resumption of differentiation. Y-SEB, immortalized in the presence of Y-27632 produces significantly more cholesterol, fatty alcohol, acids and cholesterol esters compared to SEB-1. Western blotting indicates that SEB-1 expresses both precursor and mature forms of SREBP-1; yet P-SEB and Y-SEB express only precursor form in the absence or presence of added insulin. Furthermore, treatment of SEB-1 with Y-27632 inhibits expression of mature SREBP. These data indicate that inhibition of Rho-associated protein kinase inhibits expression of SREBP-1 in human sebocytes without inhibition of overall lipid production. In addition these data suggest that ROCK inhibition may shift the balance between SREBP, PPARs or c/EBP transcription factors in mediating lipid production in human sebocytes which may be beneficial in ongoing efforts to understand the mechanisms involved in regulation of human sebum production.

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Loss of MPZL3 function results in severe skin and hair abnormalities in mice

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MPZL3 (Myelin Protein Zero-like 3) is a novel immunoglobulin-like protein abundantly expressed in the mouse skin. To investigate the role of MPZL3 in hair follicle and sebaceous gland development, we generated Mpzl3 knockout mice. Three of the six exons in the Mpzl3 gene were deleted, making it a null allele. While heterozygous Mpzl3 knockout (+/-) mice were phenotypically and histologically normal, homozygous Mpzl3 knockout (-/-) mice showed severe abnormalities in the skin and hair shortly after birth. They displayed an unkempt hair coat by two weeks of age, and severe hair loss thereafter. The hair coat that grew back during subsequent anagen remained unkempt. Histological analysis revealed sebaceous hyperplasia. Older black Mpzl3 -/- mice showed abnormal hair pigmentation, and some of them had patches of vellus-like hair. A high percentage of older Mpzl3 -/- mice also developed persistent ulcerations on the upper chest skin. Such phenotype recapitulates the skin and hair abnormalities of the rough coat (rc) mice, a natural mutant mouse strain carrying a G to A missense mutation (Arg100 to Gln) in the Mpzl3 gene. Moreover, compound heterozygous mice carrying both the rc and Mpzl3 knockout alleles showed similar phenotype. Taken together, these observations provide strong experimental evidence that MPZL3 plays an important role in hair follicle and sebaceous gland development and function, and that the mis-sense mutation in Mpzl3 is the cause of the rc phenotype. Our results also suggested that the G to A missense mutation rendered the rc allele null for Mpzl3 function, highlighting the significance of this residue for the function of MPZL3 and possibly other immunoglobulin proteins.

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Baicalin promotes hair growth

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Baicalin is known to have multiple biological functions. Here, we investigated the biological effects of baicalin for hair apparatus. We observed that baicalin significantly stimulated the transcriptional activity of pTopflash in cultured human dermal papilla (DP) cells. We also observed that baicalin increases the alkaline phosphatase (ALP) activity, an important indicator of DP activity. In addition, baicalin induced the mRNA expression of growth factors such as IGF-1 and VEGF in human DP cells. Moreover, baicalin promoted telogen to anagen transition in C57BL/6 mice. These results suggest that baicalin promotes hair growth through regulation of DP activity.

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A molecular signature for primary lymphocitic cicatricial alopecia subtypes

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Cicatricial alopecia refers to a group of disorders that result from irreversible damage to epithelial stem cells located in the bulge region of the hair follicle, usually as a result of inflammatory mechanisms. Lichen planopilaris (LPP) is the prototype of the lymphocitic subgroup. Another example of primary lymphocitic cicatricial alopecia is frontal fibrosing alopecia (FFA), first described in 1994 and currently considered as a variant LPP. Nevertheless, histopathological examination of scalp biopsies shows an increased apoptotic activity in the outer root sheath of the hair follicle in FFA as compared to LPP, and it has been suggested that FFA may selectively target androgen-dependent terminal hair follicles from the frontal scalp, leading first to miniaturization and then to destruction. Thus, we hypothesized that FFA might represent a true autonomous disease from LPP. In order to test this hypothesis, the aim of our study was to compare and validate molecular signatures for LPP and FFA. Using microarray technology, we identified gene expression patterns associated with a clinically and histologically confirmed differential diagnosis of LPP (n=3) and FFA (n=3). For microarray analysis, two biopsy samples (1 cm2 each) were collected from each patient, with one biopsy taken from the edge of an active lesion and the second biopsy from an unaffected region of the scalp. Differentially expressed genes will be validated by RT-qPCR and histological analyses, both in the original six patients and in a separate series of patients with LPP (n=10) and FFA (n=10). The results will provide a validated molecular signature for primary lymphocitic cicatricial alopecia subtypes LPP and FFA.

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Racial differences of hair steroid profiling in male pattern baldness between Korean and Caucasian populations

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The hair loss in male pattern baldness is the result of miniaturization of the hair follicle and shortening of the anagen phase of the hair growth cycle mediated by dihydrotestosterone, which is metabolized from testosterone and catalyzed by 5α-reductase. However, the androgen activities in MPB within different racial groups are not completely understood. As biological fluids demonstrate no correlation between androgen levels and MPB, steroids extracted from the hair shaft were compared in balding and normal Korean and Caucasian subjects. Human hair fibers were obtained by cutting the proximal part hair from the vertex and occipital scalp, and the 12 steroids levels were evaluated in balding and normal Korean and Caucasian subjects. The balding groups in both populations had significantly higher DHT and T levels than the control groups. Balding Caucasians had 2-fold increased epitestosterone levels compared with normal Caucasians, but only slight increases compared with normal Koreans. 5β-dihydroprogesterone levels were significantly increased in balding Koreans compared with normal Koreans, but were not significantly different from those in the Caucasian groups. Both Korean groups had higher vertex androgen levels than those of the Caucasian populations. However, levels of pregnenolone, a DHEA, and A-dione precursor were markedly higher in the Caucasian groups than in the Korean groups. For 5a-reductase, the metabolic ratio of DHT/T was increased slightly in both balding populations. The activities of 3β-hydroxysteroid dehydrogenase showed racial differences between normal and balding subjects. The quantitative results obtained from the occipital hair were not consistent with those of the vertex hair. Our findings confirm the existence of racial differences in hair steroid levels.

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Wnt5a inhibits Wnt3a-mediated signaling in human hair follicular dermal papilla cells

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Findings of recent studies have demonstrated modulation of Wnt/β-catenin signaling by Wnt5a, which is highly expressed in hair follicular dermal papilla (DP) in vivo. Here we investigated the question of whether Wnt5a can affect canonical Wnt/β-catenin signaling in DP cells. Treatment with Wnt5a resulted in attenuation of Wnt3a-mediated elevation of β-catenin signaling, which was increased by Wnt5a siRNA transfection in cultured DP cells, as examined by reporter assay. In addition, treatment with Wnt5a resulted in repressed Wnt3a-mediated expression of Axin2, EP2, and LEF1 in cultured DP cells whereas Wnt5a siRNA transfection resulted in increased Wnt3a-mediated expression of the genes in isolated DPs of cultured hair follicles. Moreover, treatment with Wnt5a resulted in attenuation of Wnt3a-mediated accumulation of β-catenin in the nucleus in DP cells. Our data strongly suggest that Wnt5a acts as an autocrine factor and attenuates canonical Wnt signaling pathway in human DP cells.

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An international repository for mouse models of skin and adnexal diseases

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The laboratory mouse is the premier mammalian biomedical model for most human diseases. The Jackson Laboratory is a private, nonprofit, research institution that developed a centralized, international repository for inbred and mutant mice in the 1940s and serves as the template for other repositories around the world. The Jackson Laboratory Rare and Orphan Disease Center was recently launched to focus on partnering with scientists, foundations, and other experts around the world to enable the development, standardization, optimization, and rapid distribution of preclinical mouse models for basic research and drug discovery. Being able to offer the resources and expertise to enable the design, construction, and management of preclinical mouse models of disease, in combination with a global delivery system, expertise in technical transfer issues, and genetic quality control, uniquely positions the Center to put new tools into the hands of scientists and thereby accelerate drug discovery. Complementing the Center's model development pipeline is one of the most comprehensive mouse repositories in the world, consisting of a growing collection of over 7000 spontaneous and engineered mouse lines including several hundred dealing with skin and adnexal diseases. The wide array of easily accessible, well-characterized tools, and mutant allele strains serves as an unmatched companion resource for the building of novel disease models with applications in translational research. Contributing your disease model to the Rare and Orphan Disease Center enables researchers across the globe to have greater access to tools for drug efficacy testing and discovery. If you would like to donate your mouse strain to the Jackson Laboratory Mouse Repository, please see: www.jax.org/donate-a-mouse. To learn more about The Jackson Laboratory Rare and Orphan Disease Center, please visit our website at www.jax.org/rare.

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R164C mutation in FOXQ1 H3 domain affects formation of the hair medulla

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A number of single gene mutations in laboratory mice produce hair follicle defects that result in severely deformed hair shafts. Occasionally mutant mice have hair shaft abnormalities that affect the cortex and/or medulla of the shaft that do not cause obvious damage but change the way the hair reflects light resulting in very subtle changes. The radiation induced satin mutant mice (SB/LeJ-Foxq1sa) have a satin-like sheen to their hair and dilute coloration. This sheen is due to failure of the hair shafts to form normal medullas. This mutation co-segregates with the lysosomal trafficking regulator (beige; Lystbg) mutation which causes pigment clumping. The original mouse satin mutation (Foxq1sa) was found to be due a 67 base deletion followed by two downstream base changes (GA to AT) in the single exon of the forkhead box Q1 gene. A further mutation in this gene (Foxq1sa-e1) arose in a mutagenesis project. A new allelic mutation arose spontaneously in the albino (Tyrc) MRL/MpJ-Faslpr colony maintained at The Jackson Laboratory. These latter mice also had a hair medulla defect. Using a complementation assay they were found to be allelic with Foxq1sa. The new mutation was found to be a C to T transition at position 490 in the Foxq1 gene (Foxq1sa-J). We report here the phenotype and molecular defect of this new allelic mutation and show the utility of these mutations as tools for studying the development of the hair shaft.

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Vitamin C inhibits the expression of inflammatory cytokines in cultured sebocytes

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Acne vulgaris is one of the most common inflammatory skin disorders caused by inflammatory cytokines. It has been established that many bioactive markers, such as inflammatory cytokines, matrix metalloproteinases (MMPs) and antimicrobial peptides (AMPs), are expressed in sebocytes. Vitamin C tends to break down in cosmetic formulations resulting in a brownish discoloration. Magnesium ascorbyl phosphate (MSP) represents a stable precursor of vitamin C that ensures a constant delivery of vitamin C into the skin. Anti-inflammatory and antioxidative effects of vitamin C may act to prevent the inflammatory acne. Our study was conducted using cultured human scalp sebocytes to evaluate changes in the expression of inflammatory biomarkers after treatment with vitamin C, MSP. Reverse transcription-polymerase chain reaction, enzyme linked immunosorbent assay and western blotting were performed in cultured sebocytes before and after treatment with MSP (10-2 mol). In addition, they were done in cultured sebocytes before and after treatment with LPS and a combination of MSP and LPS. The concentration of MSP was determined by MTT assay. The expressions of inflammatory cytokines [interleukin-1 (IL-1), IL-6, IL-8, tumor necrosis factor- α (TNF-α)], MMPs and AMPs [psoriasin, human β-defensin (hBD)-2, hBD-3 and LL-37] were changed in the level of gene and protein. Particularly, the expression of IL-1β was significantly suppressed after treatment with MSP. In conclusion, vitamin C, which is known as an antioxidant agent, may be a good effective agent to inhibit inflammatory reaction in acne.

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Novel *ex vivo* preparation to study afferent response pharmacology of guard hair follicles of hairy skin

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Our studies suggest synaptic-like vesicles (SLVs) in mechanosensory nerve terminals are part of a ubiquitous secretory system to control mechanical sensitivity. We initially used isolated rodent nerve-muscle preparations, correlating SLV turnover (FM1-43 fluorescence changes) with stretch-evoked electrical activity (Bewick et al, 2005). We then extended the SLV recycling studies to palisade endings of mouse guard hair follicles in ear (pinna) skin (Singh et al, 2009). Here we report developing the skin preparation to record palisade afferent electrical activity. Adult mice were humanely killed (ASPA, Schedule 1) and the external auditory meatus cartilage and the innervation (trigeminal mandibular branch, MDV) of the concave (anterior) aspect of the pinna skin cleared. The pinna was removed and pinned flat, anterior skin down, in carbogenated saline. Posterior skin and cartilage were removed and the MDV branches (~2) drawn into a glass suction electrode. Clearing adipose tissue exposed the follicle bases for observation or drug/FM1-43 application. Indifferent suction-electrode impedance was balanced with areolar connective tissue and the bath earthed to the recording-electrode cable screen. Electrical activity was differentially amplified and filtered (0.2 - 2 kHz) before recording (CED 1401micro interface/Spike 2 software). Spike 2 controlled a piezoelectric actuated, fire-polished, glass capillary to move 1-5 hairs. The pinna edge was reflected to access hairs, leaving a saline-filled gap between apposed skin layers. Preparation advantages are: no down hairs; well-spaced guard hairs with palisade endings but few, if any, other low-threshold endings; little spontaneous activity, so evoked responses are readily detected. We confirm guard-hair palisade ending firing is rapidly adapting and locked to specific phases of sinusoidal movements. We are now assessing candidate channels and neuromodulators influencing mechanosensitivity.

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Comparison of the young and adult rat models in chemotherapy induced alopecia

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Chemotherapy induced alopecia (CIA) is one of the most common adverse outcomes of cancer treatment. In the process of developing prophylactic and curative treatments for this distressing side effect, several rodent models have been established. Among them is a young rat model, which was used to establish dose regimens of common antineoplastic agents to induce total body hair loss. In this model, chemotherapy can be administered on days 7-13 after birth. More recently, our laboratory has developed the first pigmented, adult rat model for CIA. Rats are clipped on the dorsum on day 21 to synchronize the hair follicles (HFs), and chemotherapy is started fifteen days later. In this study, we sought to compare the functional, clinical, and histological features of the young and adult Long-Evans rat models to determine if one is better suited for CIA investigation. Rats from both age groups were randomized and treatment groups received intraperitoneal injections of Etoposide or Cyclophosphamide. Alopecia was scored 10 days post chemotherapy and dorsal skin biopsies were taken for histological analysis. Histological profiles of the hair follicles post-chemotherapy in both models demonstrated hair follicle dystrophy. In response to chemotherapy, the young rat model demonstrated complete body alopecia. In contrast, the adult rat model developed alopecia restricted to the clipped area. Additionally, the young rat model offered easier handling, required significantly less chemotherapeutic agent to achieve alopecia induction, and did not necessitate manual HF synchronization. Lastly, we found that the inherently synchronized hair cycle of the young rat provided a wider margin of time to induce CIA. In conclusion, these findings demonstrate that the young rat model may be more convenient for CIA studies.

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A position effect on FGF13 underlies X-linked congenital generalized hypertrichosis

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Hypertrichosis describes all forms of excessive hair growth for a given body location of an individual that does not depend on androgen stimulation. We and others have defined position effects involving the TRPS1 and SOX9 genes underlying autosomal forms of hypertrichosis, however, the genes that control increased hair follicle (HF) growth in X-linked hypertrichosis (XLH) remain unknown. Here, we analyzed the DNA from a Mexican family with X-linked congenital generalized hypertrichosis (MIM307150) as well as deafness and dental anomalies. Using whole genome sequencing and SNP oligonucleotide microarray analysis, we identified a 389 kb intrachromosomal insertion at an extragenic palindrome site on chromosome Xq27.1 that completely cosegregates with the disease, and confirmed it using FISH. Quantitative RT-PCR revealed that among the six genes surrounding the insertion, FGF13 levels are significantly ($p<0.001$) decreased in the patients by 4-fold, whereas mRNA levels of the neighboring genes remain unchanged. Importantly, RNA sequencing confirmed the selective decrease on FGF13 expression in XLH. We localized FGF13 to the outer root sheath (ORS) of the human HF using in situ hybridization and immunofluorescence staining, and revealed a striking decrease in FGF13 localization throughout the ORS of patient HFs. Since FGF13 lies ~1 Mb away from the insertion in XLH, we postulate that a position effect occurs as a result of the insertion and suggest that altered FGF13 levels influence important downstream signaling pathways that ultimately lead to the terminal hair overgrowth phenotype of XLH.

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Lectins as investigative tools of skin and hair follicle compartments

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The aim of the present study was to explore the distribution of glycan-conjugates in the different compartments of human skin and hair follicle. We evaluated glycans expression and diversity on frozen skin and scalp sections by FITC-conjugated lectins binding. The binding patterns of a set of fluorescent-conjugated lectins specific to different carbohydrate structures demonstrated that skin compartmentalization and epidermal differentiation were accompanied by modifications of cell surface carbohydrate moieties. For example Wheat Germ Agglutinin (WGA, specific to N-Acetyl Glucosamine) and Peanut Agglutinin (PNA, specific to galactose) decorated all epidermal layers and epidermal suprabasal layers, respectively, whereas Ulex Europeus Agglutinin (UEA, specific to fucose) only decorated stratum granulosum. In addition, Pisum Sativum Agglutinin (PSA, specific to mannose) binding appeared to be much stronger in papillary dermis. With respect to human hair follicle, WGA decorated both outer- (ORS) and inner (IRS) root sheaths (and, to a lesser extent, the keratogenous zone. UEA and Phaseolus Vulgaris agglutinin (PHA, specific to complex structures) only decorated IRS and the microvasculture while PSA binding was restricted to the hair follicle connective compartment, including connective tissue sheath and dermal papilla. Finally, Sambucus Nigra (SNA) and Maackia Amurensis (MAL-II) lectins, specific to alpha 2-6 and alpha 2-3 sialic acid, respectively revealed very different binding patterns. While the former only bound to epidermal basal layer and Langerhans cells, the later decorated both epidermal and dermal skin compartments. Alltogether, these results reveal a highly different and skin/hair follicle compartment dependent distribution of glycan-conjugates and underline that glycobiology may play a key role in skin and hair follicle physiology and homeostasis.

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Polycomb subunits Ezh1 and Ezh2 regulate the Merkel cell differentiation program in skin stem cells

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While the Polycomb complex is known to regulate cell identity in ES cells, its role in controlling tissue-specific stem cells is not well understood. Here we show that removal of Ezh1 and Ezh2, key Polycomb subunits, from mouse skin results in a marked change in fate determination in epidermal stem cells, leading to an increase in the number of lineage-committed Merkel cells, a specialized subtype of skin cells involved in mechanotransduction. By dissecting the genetic mechanism we showed that the Polycomb complex restricts differentiation of epidermal stem cells by repressing the transcription factor Sox2. Ablation of Sox2 results in a dramatic loss of Merkel cells indicating that Sox2 is a critical regulator of Merkel cell specification. We show that Sox2 directly activates Atoh1, the obligate regulator of Merkel cell differentiation. Concordantly, ablation of Sox2 attenuated the Ezh1/2-null phenotype, confirming the importance of Polycomb-mediated repression of Sox2 to maintain the epidermal stem cell state. Together these findings define a novel regulatory network by which the Polycomb complex maintains the stem cell state and governs differentiation in vivo.

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Indispensable roles of BNIP3, an inducer of autophagy, in both differentiation and maintenance of epidermal keratinocytes

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Recent studies have revealed that autophagy, a lysosomal degradation pathway, is involved in differentiation of erythrocytes, lymphocytes, and adipocytes. Keratinocyte differentiation is also going along with activation of lysosomal enzymes and organelle clearance, expecting the contribution of autophagy in this process. Our data showed that autophagosome formation was observed in the granular layer of human epidermal equivalent reconstituted from GFP-LC3 expressing keratinocytes. We also found that BNIP3 was expressed in the suprabasal layer of mouse epidermis and reconstituted human epidermal equivalent. Forced expression of BNIP3 in human primary epidermal keratinocytes (HPEK) resulted in keratinocyte differentiation, whereas knockdown of BNIP3 had an opposite effect. Intriguingly, addition of an inhibitor of autophagy significantly suppressed the BNIP3-stimulated differentiation of keratinocytes, suggesting that autophagy is involved in the process. Moreover, we also found that overexpression of BNIP3 induced autophagy in HPEK. These data clearly suggest that BNIP3 plays a crucial role in keratinocytes differentiation by inducing autophagy. Furthermore, dead cells were increased in human epidermal equivalent from BNIP3 knockdown keratinocytes, which gave us the idea that BNIP3 is also indispensable for maintenance of skin epidermis. To test the hypothesis, HPEK were irradiated with UVB. UVB irradiation stimulated BNIP3 expression and cleavage of caspase3. Surprisingly, suppression of BNIP3 expression induced by UVB irradiation caused a further increase of the cleaved caspase3 protein level, suggesting that BNIP3 has a protective effect against UVB-induced apoptosis in keratinocytes. Overall, our data shed light on functions of BNIP3, an inducer of autophagy, in both differentiation and maintenance of epidermal keratinocytes.

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Hair follicle-associated PD-L1 regulates T cell hyporesponsiveness: A potential mechanism of immune privilege

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The immune privilege of hair follicles is well established in previous studies. However, whether cultured hair follicle cells still retain this property and the individual factors that control this process are largely unidentified. In this study, we addressed these two critical questions by comparison of the expression of immune privilege related genes at both mRNA and protein levels using quantitative real-time PCR (q-PCR) and western blot (WB) in two distinct cell types, hair follicle-derived dermal sheath cells (DSCs) and non-follicular fibroblasts (FB). Compared to FB, an upregulation of programmed cell death 1 ligand 1 (PD-L1, a 7.3 ± 3.2 fold increase, $n = 4$, $p < 0.01$), and downregulation of MHC class I molecules (HLA-A and B showed a 2.8-, 2.0-fold decrease, respectively by q-PCR) were observed on DSCs; the level of PD-L1 was increased 2.3 ± 1.2 fold tested by WB. Furthermore, in allogeneic responses by human peripheral blood mononuclear cells (PBMCs, as responders) co-cultured with DSC or FB (as stimulators), the secretion of IFNg from PBMCs was significantly reduced in the presence of DSCs (23.0 vs. 5.7 pg/ml, $p<0.01$). The activation and proliferation of T-effector- (CD8+IFNg+, 32.6% vs. 26.0%, $p<0.05$; CD8+Ki67+, 12.4% vs. 4.2%, $p<0.04$) was inhibited by 20% and 66%, respectively, indicating a hyporesponsiveness to alloantigen stimulation. In addition, this hyporesponsiveness was partially removed by knockdown of PD-L1 in cultured DSC (CD8+Ki67+, 4.2% vs. 10.4%, $p<0.05$), suggesting the requirement of PD-L1 in this inhibition. This study demonstrates that cultured DSCs exhibit immunosuppressive properties, and PD-L1 is a critical factor in determining this effect, suggesting a therapeutic potential for DSC in clinical settings.

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Circadian rhythm of autophagy in human dermal fibroblasts derived from young and aged donors

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Autophagy is a critical mechanism for cellular survival that has been shown to be required for lifespan extension. However, its activity has also been shown to decrease with age. This mechanism removes cellular by-products as a result of normal metabolism, as well as from environmentally-induced cellular damage. Autophagy is also a very effective source of energy during starvation that has been observed to follow a circadian pattern in different cell types but has never been demonstrated in human dermal fibroblasts. In this study, we show, for the first time, that autophagy activity follows a circadian rhythm in human dermal fibroblasts (from a 2-day old donor) and that its activity and rhythm are lost in aging human dermal fibroblasts (from a 67-yr old donor). RT-PCR and immunofluorescence staining of LC3B, a protein associated with the membrane of the autophagosome, were used to determine autophagy levels over an 8h period following starvation in order to synchronize cellular activities. Our results show that autophagy increased and peaked at 2h after release from starvation in the fibroblasts from the young donor. In contrast, autophagy levels in fibroblasts from the old donor did not increase and did not display any rhythm. Understanding the timing and causes of these autophagic differences may lead to better protection against cell damage in aged skin cells. Additionally, developing strategies that enable aged cells to maintain their autophagic mechanisms may help to slow down the aging process in skin.

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Scd3-iCre knockin mice: A new tool for sebaceous gland-specific gene deletion

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The Cre-Lox recombination system is an established tool to induce tissue-specific deletion or modification of target genes in mice. Up to now, regulatory sequences of genes encoding keratins or other structural proteins have been most commonly used for targeting genes in the epithelial compartment of the skin. While overall effective, this strategy has the disadvantage that several different cells types in the epidermis and in the pilosebaceous unit are targeted concomitantly, resulting in potential side effects and unspecific phenotypes. Here, we report the creation of a mouse line with sebaceous gland-specific expression of Cre recombinase. We replaced the first exon of Scd3, a gene encoding an enzyme of the Stearoyl-coenzyme A desaturase family that is expressed exclusively in sebocytes, with the cDNA for codon-improved Cre recombinase (iCre) via homologous recombination in embryonic stem cells. After obtaining germline transmission of modified stem cells, we crossed the positive offspring to the Rosa26-LacZ reporter line. Functional recombination of the reporter locus, analyzed using histochemical detection of β-galactosidase (β-gal) in adult animals heterozygous for both alleles, confirmed that Cre activity in both back and tail skin was restricted to the sebaceous glands, with no staining in the epidermis, dermis, or hair follicle. We also detected a small number of β-gal-positive cells in the intestinal epithelium, in the cerebral cortex, and in the spleen, suggesting that the expression of Scd3 is not fully restricted to the skin. Our results indicate that Scd3-iCre mice can be successfully used to drive recombination specifically in the sebaceous gland. Thus, we believe that this new mouse line will become a useful tool to advance our knowledge about the roles of the sebaceous gland in health and disease.

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Human anagen scalp hair follicles contain the enzymes to synthesise *de novo* prostaglandins and prostamides from phospholipids, prostaglandin E_2 and D_2 lipid mediators

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Hair growth disorder such as androgenetic alopecia can cause psychological problems, but it is poorly controlled. Therefore, a better understanding of the mechanisms and factors regulating hair growth is required. Prostaglandin and prostamide $F_{2\alpha}$ analogues, such as latanoprost and bimatoprost stimulate eyelash growth as a side-effect when used to treat glaucoma. The mechanism of action by which these drugs stimulate eyelash hair growth is poorly understood, but we have recently shown receptors for $PGF_{2\alpha}$ (FP) and prostamide $F_{2\alpha}$ in scalp hair follicles. This suggests that those compounds may play role in the hair follicle. To determine whether human scalp follicles express the necessary enzymes to synthesise *de novo* prostaglandins and prostamides from phospholipids; and two of the other major prostaglandins, prostaglandin E_2 (PGE_2) and prostaglandin D_2 (PGD_2) lipid mediators, RT-PCR, qPCR and electrospray tandem mass spectrometry coupled to liquid chromatography (LC/ESI-MS/MS) were carried out. Scalp hair follicles were individually microdissected, RNA and lipid mediators were extracted from non-balding scalp skin from 8 men with the correct ethical approval. Isolated scalp hair follicle expressed the enzymes to synthesise *de novo* prostaglandins and prostamides from phospholipids: NAPE-PLD, PLA_2, COX-1, COX-2, $FAAH_1$ and $FAAH_2$ (n=5); PGE_2 and PGD_2 lipid mediators 17.1920 ± 2.2 and 8.5843 ± 3.5 pg/mg of follicle protein respectively (mean ± SEM, n=3). So, scalp hair follicles express the enzymes which would enable the local synthesis of prostaglandins and prostamides from phospholipids and naturally contain PGE_2 and PGD_2 lipid mediators suggesting the involvement of prostaglandins and prostamides in normal hair growth. Further analysis of the actual roles of these paracrine mediators in hair follicle may lead to new therapeutic approaches for hair disorders.

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The efficacy and safety of minoxidil 5% combination with azelaic acid 1/5% and caffeine 1% solution on male pattern hair loss

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Androgenetic alopecia is a common pattern of hair loss.current therapies like topical minoxidil and oral finasteride have shortcomings like delayed responses and limited hair growth.In this study we evaluated the efficacy of a combined topical solution (5% Minoxidil, 1.5% Azelaic acid, and 1% Caffeine) on hair growth in comparison to a 5% Minoxidil solution or a placebo solution. The study design was as a double blinded randomized controlled trial. 71 Iranian men aged 23 years or more, with a diagnosis of androgenetic alopecia were divided into three groups. They were treated with combined solution (n= 40), 5% Minoxidil (n= 20), or placebo (n= 11) for a total period of 32 weeks. The efficacy of the treatment was evaluated every 6 weeks by Wash Test (number of hairs shed), subjective patient self-assessment, and objective dermatologist assessment. At 12th week of treatment, both combined topical and 5% Minoxidil solutions significantly reduced hair shedding in comparison to the placebo group ($p<0.05$). Also the efficacy of the combined topical solution at 12th week was as good as the efficacy of 5% Minoxidil solution at 32nd week ($p<0.05$). In both dermatologist objective assessment and patient subjective self-assessment of the combined topical solution group, moderate and marked response rates were significantly higher than two other groups($p<0.001$ vs. placebo and $p<0.05$ vs. Minoxidil 5%). Our study shows that the combined topical solution results in a faster and better clinical result in comparison to 5% Minoxidill solution or Placebo. Also the combined topical solution has the same side effect profile as 5% Minoxidil solution. We recommend using this combined solution as a treatment option in daily clinical practice.

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Female patten hair loss (FPHL) – Not always patterned!

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In females, Androgenetic alopecia (AGA) usually presents with diffuse thinning over crown (widening of the partition). Conventional Ludwig-classification underestimates early-FPHL (female pattern hair loss). FPHL could be a diffuse process, which may present without patterning. Aims: To determine the prevalence of histo-pathological evidence of AGA in Indian females with chronic diffuse telogen hair-loss, without apparent patterned hair thinning with the aid of a triple, horizontally-sectioned scalp-biopsy procedure. Methodology: 80 Indian females complaining of increased shedding of hair of >6 months duration with reduction in volume of hair but without any evidence of thinning over crown were enrolled and subjected to a triple scalp-biopsy procedure, all sectioned transversely. Diagnostic definitions were applied (Terminal hair: vellus hair ratio ≤ 4:1 was diagnostic of FPHL; ≥8:1 of chronic telogen effluvium (CTE); and ratios between 4:1-8:1 were indeterminate). Results: After analyzing 240 biopsies from 80 women, 46/80(57.5%) had FPHL, 26/80(32.5%) had CTE, and 8/80(10%) were indeterminate. Discussion: Had scalp-biopsy not been offered these 80 patients, there would have been a delay in diagnosis of AGA(in >57% of women), delaying treatment. Scalp-biopsy can also help to distinguish CTE from FPHL. Conclusion: Though scalp-biopsy serves as a diagnostic tool for early 'non-patterned FPHL', it is currently grossly underutilized. It should be offered as a routine diagnostic procedure in women presenting with hair-loss; especially with chronic hair shedding without thinning; where a clinical diagnosis of FPHL is not possible. Indeterminate group of patients should be followed up to see whether they finally evolve into FPHL.

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Multipotency can be rescued in cultured adult keratinocytes

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Freshly isolated neonatal or adult epidermis contains multipotent stem cells which can be recruited to regenerate not only hair follicles but also interfollicular epidermis. After serial passages, cultured follicular and glabrous (sole) epidermal cells are morphologically identical and show standout ability of clonal expansion. These highly passaged cultivated cells' rather small and uniform appearance, together with their strong ability to self-renew and to form holoclones, indicates that they are stem cell-like. However, these cultured epidermal cells lose track of hair induction, meaning they are unable to adopt follicular fate in response to dermal papilla (DP) signals in recombination experiments. The limited ability to differentiate makes them less alike stem cells and no longer multipotent. To address whether cultured epidermal cells can become competent toward DP signals and differentiate into follicular lineages to form hairs, we isolated and serially cultured rat vibrissa outer root sheath and sole epidermis, and overexpressed Wnt/beta-catenin signaling pathway before these cultured epidermal cells were recombined with inductive adult DP cells in hair regeneration model. Successful turn-on of Wnt signaling was proved by upregulation of both beta-catenin mRNA and protein levels in these cells. Neogenesis of hair shafts with differentiating inner root sheath and hair cuticle were observed within 4 weeks. Control groups combining inductive DP cells and non-treated cultured epidermal cells yielded negative results. Here we investigated the possibility of rescuing multipotency on cultured keratinocytes by means of overexpressing Wnt signaling pathway. These "pre-treated" or "rescued" cultured cells regain the ability to differentiate into follicular epithelium and form hairs. The findings suggest these long term cultivated keratinocytes can be rescued their multipotency, and the results provide the potential cell-based treatment for hair loss by using expansion of adult individual's own dermal and epidermal cells.

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A mouse model for *in vivo* quantitative monitoring of Wnt/B-Catenin signalling in skin reveals macroscopic hair cycle dynamics in health and disease

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Wnt signalling through its canonical pathway promotes hair follicle development and differentiation via the stabilization of nuclear beta catenin. These features have been largely described at the individual cell or hair follicle level. We aimed to generate a model for in vivo quantitative monitoring of beta catenin activity in the skin to follow wnt signalling in mouse back skin at the macroscopic level. We generated mice harbouring a luciferase reporter gene under the control of beta catenin binding sites TCF/LEF inducible promoter. Using in vivo bioluminescence imaging, we were able to track beta catenin activity in the skin from P1 in rostro-caudal waves. The signal peaked at P9 with >400 fold increase. Histological assessment allowed attributing signal levels to specific phases of the hair follicle cycle. Furthermore, we were able to reproduce all macroscopic features of hair follicle biology, such as propagating waves, border stability, refractory telogen and random initiation points. In adults, hair plucking or cyclosporine predictably induced beta catenin activity with an intense signal appearing 6 days after initiation. We took advantage of the possibilities offered by this model to assess the level of beta-catenin activity in situations of health and disease. During pregnancy, the peak of beta-catenin activity was not modified. However, there was a delay in the natural entry into anagen. Similarly wound healing and models of defective hair growth affected the level of bioluminescence. Finally, mathematical modelling of beta-catenin signalling based on a generic dynamical mechanism for producing oscillatory behavior in activator-inhibitor system allowed to reproduce the characteristic patterns of hair follicle progression and cycling in a two dimensional grid. In conclusion, tracking Wnt signalling macroscopically in the mouse back skin allows a detailed understanding of hair cycle progression and could be used for screening drugs or molecular targets.

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The human hair follicle cycle: A guide

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The hair follicle (HF) is a complex mini-organ that continuously undergoes significant transformations that constitute the HF cycle including phases of rapid growth (anagen), apoptosis-driven regression (catagen) and relative quiescence (telogen). Since HF cycling is associated with hair growth disorders, it is clinically important to recognize and classify the hair cycle phases of individual follicles present in human skin biopsies. Accurately assessing the human HF cycle requires experience and can be frustrating and confusing. While histologic guidelines for the accurate classification of the murine hair cycle exist, the field of human HF biology still needs a comprehensive guide that presents clear criteria useful for accurately and reproducibly classifying HFs according to their hair cycle phase. Here, we provide a comprehensive guide that integrates histologic features with immunohistological marker analyses. Distinguishing between vellus and terminal HFs, we suggest basic and auxiliary criteria for recognizing key hair cycle phases and illustrate these phases by schematic drawings and representative micrographs that facilitate phase recognition. The suggested auxiliary criteria/ markers include analyses of Ki-67/TUNEL+ and Masson Fontana. In addition, we show how a hair cycle phase can still be identified even if key HF structures are only partially visible. This is complemented by an illustrated glossary of HF anatomy terms. Our guide provides a set of easily applied standardized criteria to perform human hair cycle analysis and this morphology-based HF cycle classification will remain an indispensable tool for basic and applied human hair research.

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DGAT1: A new player in sebaceous gland homeostasis, sebocyte secretory droplet formation and hair follicle cycling

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Mammalian skin characteristically displays sebaceous glands (SG) whose most important role is the production and secretion of sebum to the skin surface where it contributes to thermoregulation, waterproofing and anti-bacterial defence. The final step in the formation of triglycerides, one of the main sebum components, is catalysed by Acyl CoA: diacylglycerol acyltransferase (DGAT) enzymes. DGAT1 knockout (KO) has previously been shown to cause SG atrophy in mice only after puberty, indicative of a sex-hormone trigger. In order to establish whether the effects of DGAT1 KO were present only postpubertally, the SGs of DGAT1 KO mice (C57BL/6N background) were compared to age-matched wild type mice and assessed using histological and ultrastructural methods, at post-partum weeks 1-9. Skin phenotype changes were apparent histologically from week 1, with cellular degeneration, misshapen nuclei and nuclear condensation occurring in the SGs of DGAT1 KO mice, which became more prominent with increasing age. Sebocyte size also decreased with age in KO mice, with overall sebocyte size in KO mice significantly smaller than wild type mice ($p<0.001$). Ultrastructurally, KO mice presented with fewer correctly formed sebocyte lipid secretory droplets, which persisted and became more advanced with progressing age. Qualitative hair phenotype analysis also suggests that deletion of DGAT1 accelerates spontaneous catagen entry. If confirmed by quantitative hair cycle histomorphometry, these preliminary data indicate that DGAT1 is not only involved in sebum production as well as SG function and maintenance, but may also impact on hair growth control. This introduces a triglyceride synthesis-regulatory enzyme as a novel regulator of pilosebaceous biology.

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Autophagy-related gene Atg5 controls terminal differentiation of sebocytes in the preputial gland of the mouse

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Terminal differentiation of sebocytes represents a special form of programmed cell death that is preceded by a major intracellular reorganisation and the accumulation of lipid droplets. The molecular mechanisms of this process are incompletely understood. Here, we studied the role of autophagy, a genetically well-defined catabolic process, in sebocyte differentiation. As a model, we used the preputial gland which is the largest sebaceous gland of the mouse. Mice carrying floxed alleles of the essential autophagy gene, Atg5, were crossed with mice expressing the Cre recombinase under the control of the keratin K5 promoter which is active in proliferating keratinocytes and sebocytes. The resulting Atg5 f/f K5-Cre mice were viable and had an apparently normal epidermal morphology. Isolated K5-expressing cells of these mice did not express Atg5 and consequently lacked lipidation of LC3, a critical step of autophagy. Sebocytes within autophagy-deficient preputial glands were able to form lipid droplets but showed cytoplasmic alterations with aberrant hematoxylin-eosin staining. Nuclear DNA was degraded at a premature stage of terminal differentiation when autophagy was blocked by Atg5 deletion. Strikingly, DNA fragmentation involved a lower amount of intermediates accessible to terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) than in differentiating wildtype sebocytes. These data suggest that autophagy suppresses cell death to facilitate normal differentiation of the preputial gland sebocytes.

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Oxidative stress and cell senescence in androgenetic alopecia (AGA)

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The hair follicle dermal papilla (DP) is essential for hair growth and is known to be a target tissue for androgens. However, the mechanism for decreased hair growth in AGA is unknown. We have shown that DP cells from balding scalp undergo premature senescence in vitro when compared to matched DP cells from non-balding scalp and that this is associated with up regulation of p16INK4a and down regulation of BMI-1. Patient matched DP cells from balding scalp have significantly higher levels of reactive oxygen species (ROS) undergo fewer population doublings and have higher levels of cell senescence when cultured at 21% oxygen compared to 2% oxygen and these differences correlate with changes p16INK4a and BMI-1 expression. We also show the established DHT-TGF-β secretion axis is active at 21% but not 2% oxygen. These data suggest that oxidative stress may exacerbate the onset of androgenic alopecia by affecting senescence and DHT-induced TGF-β secretion, a known inducer of catagen and inhibitor of hair follicle growth.

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Reprogramming regular skin fibroblasts into hair inducing dermal papilla cells

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Cell-based regenerative therapies are still unavailable to restore hair follicles in hair loss patients and to generate new hair follicles in burn victims or patients with other debilitating skin disorders. Currently, there is a lack of know-how to expand fully functional DP cells in the culture dish for hair inductive cell transplantations. To generate sufficient cell quantities for hair regenerative therapies we sought to reprogram regular fibroblasts into DP cells. Overexpression of previously identified DP signature transcription factors (TFs) in freshly isolated fibroblasts in combination with inhibitors of histone modifiers significantly upregulated several DP signature genes. Furthermore, 3D aggregation clustering of TF overexpressing fibroblast lines isolated from double-transgenic Sox2-GFP/Lef1-RFP reporter mice activated reporter activity and induced the DP molecular identity. Our preliminary data suggest that the right combination of DP TFs can reprogram DP niche fate in regular fibroblasts that can potentially be utilized in future hair restoration efforts.

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Mitochondrial dysfunction present early and trigger the pathogenic sequelae in cicatricial alopecia

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We previously showed that altered lipid metabolism in hair follicle cells plays a crucial role in triggering the inflammatory response in primary cicatricial alopecia (PCA). However, the mechanisms responsible are poorly understood. Global metabolomics analysis of unaffected frontal fibrosing alopecia (FFA) scalp biopsies revealed defective energy metabolism that is indicative of mitochondrial impairment. Transmission electron microscopy (TEM) of hair follicle cells from unaffected FFA biopsies showed swollen mitochondria suggesting for the first time that mitochondrial dysfunction may be an early event in disease pathogenesis. Mitochondria are the "power-houses" of the cell and generate ATP. Changes in activity of the enzymes of the mitochondrial respiratory complex (MRC) can disrupt energy production and have devastating consequences for the cell, tissue and organ. To identify the mitochondrial defect in FFA, we integrated transcriptomics and proteomics approaches. Protein changes in major mitochondrial pathways including the MRC, antioxidant systems, the TCA cycle and fatty acid metabolism were observed in FFA. The consequence of mitochondrial damage with decreased antioxidant defense capacity is the generation of Reactive Oxygen Species (ROS) that can further damage the mitochondria. Immuno-blotting with 3 nitrotyrosine and 4 hydroxy 2 nonenal antibodies followed by mass spectrometric analysis of the major bands identified from unaffected FFA showed oxidative modification of the MRC and the ubiquitin-proteasome system (UPS). Impairment of UPS causes the accumulation of potentially harmful modified proteins that can further damage the mitochondria. Functional studies validated the high through put analysis and confirmed that all five mitochondrial complexes are defective in FFA. Our data shows for the first time that early mitochondrial dysfunction followed by oxidative stress and an impaired UPS elicit the pathogenic sequelae in PCA. Restoring mitochondrial function may be a promising new therapeutic strategy for PCA.

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Human hair follicle neogenesis using microenvironmentally reprogrammed dermal papilla cells

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Hair follicle (HF) neogenesis refers to the generation of an entirely new HF in recipient skin using HF dermal papilla (DP) cells. This has been extensively demonstrated in rodent skin, either using intact DP, or cultured DP cells. In stark contrast, however, HF neogenesis in human skin has not previously been achieved using human cells. We previously performed global transcriptional profiling of both intact and cultured DP cells using the Affymetrix U133 Plus 2.0 array, which revealed several pathways expressed in intact DP, which are capable of neogenesis, but absent in cultured cells, that lack the potential to induce *de novo* HF growth. We postulated that one approach to restoring the 3D microenvironmental and anatomical context of intact DP is to grow the cells in hanging drops, which results in the formation of DP spheroids. We then profiled DP spheroids for changes in gene expression, and determined that the average correlation coefficient between the transcriptomes of intact DP vs cultured cells is 0.42 while between intact DP vs spheroids it is 0.56, which equates to a significant restoration of an intact DP signature by 3D culture. To evaluate if recapitulation of the DP signature equated to a restored inductive potential, we established a contextual human-to-human HF neogenesis assay that could be used to assess the inductive capacity of human DP cells in human skin. When we micro-implanted DP spheroids into recombined foreskins placed onto the back of SCID mice, we observed dramatic HF neogenesis by 6 weeks. HFs grew down into the dermis and produced hair fibers, showing for the first time that intact human DP can induce a *de novo* human HF. Thus, we show that the inductive potential of human DP cells can be restored by growth in spheroids, which can induce entirely new HFs in human skin. We conclude that the partial restoration of the transcriptional profile in human DP cells, achieved simply by growing the cells in a 3D spherical microenvironment, is sufficient in some instances to restore the inductive capacity of DP cell cultures and elicit complete human HF neogenesis.

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Trichomegaly, or Hollywood "movie lashes", resulting from mutations in FGF5 and an elongated hair cycle anagen phase

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Trichomegaly, or Hollywood "movie lashes" (OMIM 190330), refers to an autosomal recessive disorder characterized by the growth of excessively long eyelashes, however, the genetic basis of this disease remains unknown. To identify molecular regulators of eyelash growth, we ascertained two consanguineous families from Pakistan that presented with familial trichomegaly. First, using whole genome SNP genotyping and autozygosity mapping, we identified an extended region of high autozygosity on chromosome 4q21.21 within family 1. We then used whole exome sequencing and identified distinct pathogenic mutations in both families within the fibroblast growth factor 5 (FGF5) gene (c.158_159delTA and c.459+1delG), which lies directly within the region of autozygosity. Subsequent direct sequencing of FGF5 in several additional trichomegaly families identified a third mutation (c.T520C). To ascertain the effect of FGF5 mutations on human hair growth and anagen duration, we obtained and measured plucked hair fibers from patient forearms, and found them to be significantly longer than control hair fibers, indicative of a prolonged hair growth phase and a shifted anagen:telogen ratio. Moreover, we found that FGF5 protein was absent in plucked patient hair samples compared to controls using whole mount immunofluorescence. Mutations in FGF5 underlie the *angora* phenotype in several mammalian species including mouse, dog and rabbit among others, which all exhibit an elongated anagen phase and excessive hair growth, however, until now a human counterpart had not been described. We have identified FGF5 as a crucial regulator of hair growth in humans for the first time, and demonstrated a profound effect on the elongation of hairs usually residing in telogen. Moreover, since this phenotype is strikingly visible in the eyelashes, this discovery raises the possibility of therapeutic targeting of FGF5 to selectively enhance eyelash growth.

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Impaired ubiquitin-proteasome system in cicatricial alopecia: Implications for inflammatory response

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Primary Cicatricial Alopecia (PCA) is a group of inflammatory disorders that cause scarring and permanent hair loss. Mechanisms that trigger the inflammatory response in PCA are poorly characterized. Here, the goal was to determine whether the ubiquitin-proteasome system (UPS), a major ATP dependent protein degradation pathway, is defective in PCA. The UPS consists of concerted actions of enzymes that tag ubiquitin onto proteins to mark them for degradation. This leads to their recognition by 26S proteasome complex that degrades ubiquitinated proteins. TEM studies of PCA subtypes showed the accumulation of protein aggregates in hair follicle cells. Studies aimed to identify protein aggregates tagged or untagged with ubiquitin from diseased cells are currently in progress. Evidence of protein aggregation suggests for the first time that the proteasome system may be impaired in PCA. We used an integrated proteomics and transcriptomics approach to characterize the UPS in centrifugal cicatricial alopecia (CCCA), frontal fibrosing alopecia (FFA), and lichen planopilaris (LPP). Our analysis revealed that the gene and protein expression associated with the proteasome complex are de-regulated in unaffected scalp tissue in all subtypes. In addition, enzymes required for protein ubiquitination are de-regulated suggesting the impairment of the UPS in PCA. To determine if oxidative stress causes UPS inactivation, we carried out western blotting with 4 hydroxynonenal antibodies. Mass spectrometric analysis of the bands obtained showed that enzymes of the ubiquitin-proteasome complex are subject to oxidative modification, suggesting that this post-translational modification causes impairment of UPS. Intriguingly, proteome analysis identified the major histocompatibility complex (MHC) and inflammasome proteins to be de-regulated in PCA. Our data suggests that accumulation of damaged proteins due to UPS impairment may serve as the primary trigger for inflammatory responses in PCA. Thus, UPS activation may be a promising new treatment strategy for PCA.

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Continuous systemic low-dose steroids as a therapy option for alopecia areata of childhood

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Alopecia areata (AA) is the most common cause of inflammation-induced hair loss and is associated with an increased overall incidence of autoimmune disorders. AA is hypothesized to be an organ-specific disease mediated by T-lymphocytes directed to hair follicles and is possibly triggered by environmental factors on the basis of a genetic predisposition. We report on 5 year-old Caucasian female twins who presented with a 12-month history of AA universalis and a positive family history for atopic dermatitis and AA. Previous treatment with topical methylprednisolonaceponate was unsuccessful. The laboratory examination revealed anti-thyroid peroxidase and anti-thyreoglobulin antibodies. In response to the enormous social pressure we began a systemic therapy with glucocorticosteroids (GCS), starting with a dose of 2 mg prednisolon per kilogram body weight. Dosage was tapered over 8 weeks until reaching the body weight-depended Cushing level (2.5 mg/die). Hair started to re-grow in week 4 and dosage was further reduced to 1 mg a.d. The children achieved complete remission and GCS therapy was discontinued after a total of 12 months. In the process of teething, 4 months after treatment end, the girls simultaneously developed small bald patches. Systemic GCS therapy was restarted at the individual Cushing level and tapered to 1 mg a.d. This led again to a disease-free state in both patients. In response to the parents' request, GCS treatment was discontinued after a total of 12 months, which caused a relapse after 4 months. GCS therapy was restarted once more according to the previous protocol. Our results demonstrate that continuous low dose GCS therapy of AA patients can induce a complete suppression of disease activity without causing any neuroendocrinological side effects. Further, the observation that every intended treatment interruption was followed by a disease recurrence makes us believe that hair growth in our AA twins was a consequence of steroid administration and not due to a placebo effect. Clearly, well designed and controlled studies are needed to validate this assumption.

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Epidermal insulin/IGF signalling is a crucial regulator of epidermal stratification and UVB-induced skin responses

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The epidermal barrier is established through a stratification program, which is accompanied by a shift from symmetric towards asymmetric divisions (ACD). We identified cell autonomous insulin/IGF (IIS) signalling as crucial regulators of this process. Loss of epidermal IIS leads to a biased loss of ACD resulting in impaired stratification. Upon loss of IIS, the FoxO transcription factor is retained in the nucleus, where it binds and inhibits p63-regulated transcription. Accordingly, knockdown of FoxO1/3 in IIS deficient keratinocytes abrogates this inhibition, and, importantly, transgenic expression of constitutive nuclear FoxO in the epidermis abrogates ACD and stratification. Persistent transcription blocking DNA lesions attenuate IIS signalling. As UV-induced persistent DNA damage is a primary cause for carcinogenic mutations and increased IIS signaling is associated with skin cancer we asked if epidermal IIS signalling regulates UVB responses in the skin. IGF-1Repi-/- mice were more susceptible to UVB-induced apoptosis but, surprisingly, these mice later showed an enhanced and sustained hyperproliferative response upon UVB radiation, in contrast to its role in stratification, where loss of IIS counteracts growth and proliferative potential. Further analysis revealed that this hyperproliferation is a non-cell autonomous effect and might be driven by an altered inflammatory response. At present we are examining this in more detail and ask if IIS dependent regulation of FoxOs are involved in UVB skin responses. Taken together, the data reveal a critical role for IIS in FoxO-dependent control of p63 activity in epidermal stratification and identify IIS signaling as an important mediator of UVB responses in the skin.

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Blocking K_{ATP} channels in scalp follicles in organ culture inhibit hair growth and alter paracrine signalling increasing inhibitory TNF and decreasing stimulatory FGF-10

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Minoxidil, a common alopecia treatment, opens SUR2 K_{ATP} channels in plasma membranes and stimulates hair growth. Human hair follicle contains two types of K_{ATP} channels, SUR1 and SUR2B, with differing sensitivity to K_{ATP} channel regulators. Understanding their mechanisms may also be useful in excessive hair growth disorders, hirsutism and hypertrichosis. Therefore, we wished to clarify the effect of closing K_{ATP} channels in human hair follicles. Since tolbutamide can close both SUR1 and SUR2B channels, we assessed its effect on hair growth and paracrine signalling in scalp hair follicles in organ culture. Scalp follicles were microdissected and cultured with, or without, tolbutamide. Follicles were observed, measured and photographed daily for 9 days in 10nM-1mM. Total RNA was also extracted after 4 days incubation with 1mM tolbutamide, (3 pooled samples, each from 5 individuals), and differences in gene expression determined using DNA microarray analysis; quantitative real-time PCR confirmed differences. Tolbutamide (100nM-1mM) significantly reduced the daily increase in follicle length, percentage of follicles remaining in anagen and overall increase in hair growth; it had no effect at 10nM. Tolbutamide at 1mM significantly altered paracrine signalling system, increasing gene expression of inhibitory TNF ($P<0.05$) and its receptor, TNFRSF1A ($P<0.01$) and decreasing stimulatory FGF-10 ($P<0.01$) and FGFR2 receptor ($P<0.05$). Closing both SUR1 and SUR2B K_{ATP} channels, or SUR1 only, decreased hair growth, induced catagen-like changes and altered paracrine signalling. This is strong evidence for an important biological role for follicular K_{ATP} channels in hair growth. Greater understanding should facilitate novel therapeutics for hair disorders.

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MicroRNA-214 controls skin and hair follicle development by targeting beta-catenin and modulating the activity of Wnt signaling pathway

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Skin development is governed by complex programmes of gene activation and silencing, including microRNA-dependent modulation of gene expression. We show that miR-214 is expressed in the epidermis and hair follicle (HF) during skin morphogenesis. To explore its role in the control of skin development we generated doxycycline-inducible miR-214 (K14-rtTA/TRE-miR-214) transgenic mice (TG). Activation of transgene expression during skin embryogenesis resulted in the development of thinner epidermis, reduced keratinocyte proliferation and appearance of a "rough" coat postnatally as the result of the development of about 40% fewer HFs in back skin. The hair bulbs in TG mice were dramatically reduced in size, which was associated with decreased cell proliferation in the hair matrix and significantly thinner hair shaft production. However, the ratio between different HF types (guard, awl, auchene or zig-zag) in TG and WT mice was not changed. The inhibitory effects of miR-214 on skin and HF development were associated with decreased expression of the key components of Wnt signalling pathway (beta-catenin, Lef-1), as well as with activation of BMP signalling in the epidermis and HFs as was documented by increased pSmad1/5 expression. Luciferase reporter assay confirmed bio-informatic prediction that miR-214 directly targets beta-catenin in keratinocytes. In primary keratinocytes, miR-214 mimic prevented nuclear translocation of beta-catenin in response to Wnt activator lithium chloride, abrogated lithium chloride-induced expression of Axin2, and significantly diminished TOPflash activity induced by the Wnt activator BIO. Taken together, these data reveal an essential role of miR-214 in the control of skin and HF development and suggest miR-214 as a key regulator of the activity of Wnt/beta-catenin signalling pathway in the keratinocytes.

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Embryonic-like cell-secreted proteins induce hair growth in a phase I/II trial in male pattern baldness

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A Phase I/II clinical trial was performed to study the safety and efficacy of a bioengineered human cell-derived formulation, termed Hair Stimulating Complex (HSC), in stimulating hair growth in subjects with male pattern baldness. HSC contains naturally secreted growth factors known to be important in hair growth, including Follistatin, KGF, and VEGF. The clinical study was a double-blind, randomized, two center trial in 56 subjects. All subjects tolerated the eight 0.1cc intradermal injections at baseline and 6 weeks well, and no signs of an adverse reaction were reported. Blood and urine samples taken before and after each injection set showed no liver, kidney, or bone marrow toxicity. Trichoscan image analysis of treated sites were taken at baseline, and 12, 24, 36, and 48weeks. At the 12 week time point significant improvements in total ($p=0.0013$), terminal ($p=0.0135$) and vellus ($p=0.033$) hair growth over baseline was seen as was an increase in cumulative thickness density ($p=0026$). The primary efficacy endpoint of increased terminal hair at 12 weeks was met, with a 19.5% increase seen, a 49.5% increase over the same endpoint in our proof-of-concept trial. In addition, unlike currently approved products, HSC induced hair growth in the temporal recession as well as vertex and mid scalp regions, and was highly effective in men over 40 years of age. At the 48 week time point there continued to be a significant increase in total hairs over baseline ($p=0.028$). These results clearly demonstrate the safety and efficacy of intradermal injections of HSC in subjects with androgenetic alopecia.

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Polycomb component Cbx4 inhibits the expression of non-epidermal lineage genes and regulates cell proliferation in keratinocytes during skin development

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During epidermal development, lineage-specific programmes of gene expression are tuned for activation of keratinocyte-specific genes, while non-epidermal genes become repressed. Cbx4 is a component of the Polycomb repressive complex regulating gene expression programmes in many cell types including epidermal stem cells and thymic epithelial cells. In the developing skin, Cbx4 is expressed in basal and suprabasal epidermal keratinocytes, while its expression is strongly decreased in the skin epithelium of p63 knockout mice. p63 binds Cbx4 promoter region in keratinocytes and positively regulates Cbx4 in the reporter assay, thus suggesting Cbx4 as a direct p63 target. Cbx4 knockout embryos show a marked decrease in the epidermal thickness and reduced keratinocyte proliferation, the characteristic features of the skin phenotype of p63 knockout mice. Analyses of gene expression after Cbx4 ablation in keratinocytes revealed an activation of expression of numerous non-epidermal lineage genes, such as neuronal and mesodermal-specific genes. In addition, expressions of the cyclin-dependent kinase inhibitors p19 and p57 were markedly increased in the epidermis of Cbx4 null mice, suggesting that Cbx4 might be involved in mediating the effects of p63 on cell cycle-associated genes in epidermal keratinocytes. Taken together, these data strongly suggest that Cbx4 represses non-epidermal lineage genes and regulates cell proliferation in keratinocytes. Thus, Cbx4 serves as an important component of the p63-regulated program of gene expression, which includes not only the establishment and maintenance of expression of the epidermis-specific genes in keratinocytes, but also involves the repression of non-epidermal lineage genes.

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p63 transcription factor controls the expression of nuclear envelope components and organization of nuclear architecture during epidermal development

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During tissue development, multi-potent progenitor cells establish lineage-specific gene expression programmes, leading to differentiation into specialized cell types. In the developing epidermis, p63 transcription factor controls the expression of a large number of genes that constitute epidermal differentiation program, as well as regulates expression of the chromatin remodellers, such as Satb1, to establish a proper spatial arrangement of its target genes in the nucleus. Here we show that in p63-null mice, as well as in primary mouse keratinocytes treated with p63 siRNA, about 20% of nuclei showed altered morphology, compared to the corresponding controls. Alterations in the nuclear shape seen in p63 null mice were accompanied by the decreased expression of the nuclear lamins (Lamin A/C and Lamin B1), proteins of the LINC complex (Sun-1, nesprin-2/3) and Plectin, which links nuclear envelope with the cytoskeleton. Furthermore, ChIP-qPCR assay in adult mouse epidermal keratinocytes showed p63 enrichment on the promoters of the Plectin-1c, Sun-1 and Nesprin-3 genes, suggesting them as possible direct p63 targets. Alterations in the nuclear shape in p63-null mice were also accompanied by the loss of the peripheral distribution of the heterochromatin proteins 1alpha and gamma, heterochromatin-associated repressive histone modification H3K27me3, as well as by the reduced expression of the Polycomb proteins Ezh2, Cbx4 and Ring1b. Taken together, these data demonstrate that p63 regulates keratinocytes gene expression program not only through the regulation of adhesion molecules, cytoskeleton proteins and chromatin remodelling factors, but also through the components of the nuclear envelope, thus suggesting an existence of the functional links between the cytoskeleton and three-dimensional nuclear organization.

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Blockade of Gli transcriptional activity in mature anagen hair follicles leads to impaired matrix cell proliferation, aberrant differentiation, and defective hair shaft formation

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Treatment with Smo antagonists blocks oncogenic Hedgehog (Hh) signaling and leads to basal cell carcinoma (BCC) regression, and in many patients, development of alopecia. Physiologic Hh signaling is required for the normal transition from a resting telogen follicle to a growing anagen hair follicle. The Hh pathway remains active in outer root sheath and hair matrix cells of mature anagen follicles, but little is known of its functions during this phase of the hair cycle. We have begun exploring this issue using mice carrying a doxycycline-inducible dominant-negative Gli2 allele, Gli2ΔC4, to inhibit Hh pathway-driven Gli transcriptional activity. To assess blockade of Hh signaling activity at the single cell level, we included a *Gli1-lacZ* allele and identified cells expressing the Hh target gene Gli1 by staining for β-galactosidase. We confirmed that Gli2ΔC4 is a potent inhibitor of hair follicle growth when induced prior to anagen initiation, in keeping with previous data establishing a requirement for Hh signaling activity at early stages of anagen. We then activated Gli2ΔC4 expression between 9 and 14 days after depilation to examine effects on growing hair follicles in mature anagen. Hh signaling activity (Gli1 expression) in hair matrix cells was strongly inhibited in mice expressing Gli2ΔC4. This was associated with a marked reduction in Ki67+ matrix cells, precocious appearance of inner root sheath and hair shaft lineages in the proximal hair bulb, ectopic expression of epidermal keratins K1 and K10, and ultimate formation of misshapen and markedly shorter hairs. The striking alterations in maturing anagen hair follicles expressing Gli2ΔC4 argue that Hh pathway-mediated Gli transcriptional activity plays an essential role in coordinating hair follicle growth and maturation throughout anagen, which may account for the relatively rapid appearance of alopecia in BCC patients treated with Smo antagonists to block Hh signaling.

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"Weed against zit?" – Investigation of the anti-acne effects of cannabidiol

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We have previously shown that the non-psychotropic phytocannabinoid cannabidiol (CBD) qualitatively and quantitatively normalized the "pro-acne agents" (e.g. arachidonic acid) induced lipogenesis of human SZ95 sebocytes, via the activation of transient receptor potential vanilloid-4 (TRPV4) ion channels. In our current study, we aimed at further dissecting the putative additional "anti-acne" effects of CBD and exploring the related intracellular signaling pathways. CBD exerted a remarkable "universal" anti-inflammatory effect (complete normalization of the elevated tumor necrosis factor-α expression induced by "pro-acne agents" or Toll-like receptor activators) and an anti-proliferative action as well, both in vitro and ex vivo (full thickness human skin organ culture). CBD did not modify the activities of multiple intracellular signaling pathways (e.g. PP2B, PKA, PKC, and PI3K). However, CBD was found to inhibit the anandamide (an important endogenous lipogenic agent) induced activation of ERK1/2 MAPK in a TRPV4-dependent manner. Moreover, a genome-wide microarray analyses (as well as confirmatory RT-qPCR), performed on three independent sets of control and CBD-treated samples, revealed that down-regulation of NRIP1 and up-regulation of TRIB3 (well-known regulators of the lipogenesis and lipid storing) might be the downstream effectors in mediating the actions of CBD. Taken together, our results demonstrate that CBD exert a unique, "triple" anti-acne activity (lipostatic, anti-proliferative and anti-inflammatory actions) in vitro and ex vivo as well. Therefore, CBD and possibly other modulators of the identified signaling pathways might be powerful novel tools in the treatment of acne vulgaris.